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Original Article

Xenotransplantation of pancreatic islets microencapsulated in agarose gel

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Abstract

Prevention of rejection is critical to achieve successful pancreatic islet transplantation. Protection of islet cells from rejection by isolating the islets in artificial membranes has been used instead of immunosuppression treatment. In this study we investigated the microcapsulation of hamster islets in hydrophilic microcapsules made of agarose. The microcapsulated hamster islets were placed intraperitoneally in mice in which diabetes was induced by a single dose (150 mg / kg of body weight) of streptozotocin. Five groups were studied. The first group (5 mice) received free hamster islets (1000 islets). The second group (5 mice) received 1000 empty agarose microcapsules and 1000 free hamster islets. The third group (10 mice) received hamster islets microencapsulated in agarose (500 microcapsules). The fourth group (10 mice) received 1000 islet microcapsules. The fifth group (10 mice) received 1000 islet microcapsules cultured in CMRL - 1066 medium for 4 weeks at 37 ^{0}C. Mice of group 1 and group 2 failed to achieve normoglycemia. Recipient mice received microencapsulated islets (group 3,4,5) maintained normoglycemia for a mean of 45 ± 5 days (range 30 – 65 days). These cured mice had normal glucose tolerance tests, which indicates that islets in the microcapsules were functioning as if they are in an intact pancreas. Microcapsules, retrieved up to 30 days after transplantation, showed no evidence of tissue reaction. Our study indicates that agarose microcapsules can protect islet xenografts from rejection. These microcapsules may be suitable for human clinical islet xenotransplantation.

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Key words: Xenotransplantation, agarose, micro-capsules

Introduction

Pancreatic islet transplantation for the treatment of diabetes has been limited by the inability to prevent islet rejection. Various approaches for preventing islet graft rejection, and thus maintaining long term islet cell function have been investigated [1]. One of these approaches is the protection of the transplanted islets from recipient immune system by enclosing them in membranes that prevent inward diffusion of immune mediators, but allow free exchange of glucose and insulin [2]. Previous studies had demonstrated that islet can be entrapped in viable state in alginate capsules which are characterized by a shell of alginate-polyethyleneimine [3]. The drawback of this technique is the fragile nature of such preparations and the instability of these microcapsules [4,5]. When these microcapsules were placed intraperitoneally they produced only temporary remission of hyperglycemia [6]. Another study showed that encapsulation of human islets in acrylic-copolymer fiber could prevent rejection of the grafts without immunosuppression for a period of two weeks after subcutaneous implantation [7]. Also, Iwata et al [8] reported the advantage of agarose for microencapsulation of islet allograft and xenograft. In these studies allograft survival with maintenance of normoglycemia was achieved. Our study was conducted to examine whether hamster islets enclosed in agarose microcapsules can survive in the peritoneal cavity of mice as xenograft transplantation. Also to examine whether these microcapsules can maintain normoglycemia and provide normal glucose tolerance test responses.

Material and methods

Animals: Golden Syrian hamsters, weighing 100 – 120 g were used as islet donors. Swiss mice were the recipients. Non fasting plasma glucose levels of the recipient mice were determined before the induction of diabetes. Blood sugar levels were monitored via orbital sinus blood samples with Aqua trend sensor. The mice were made diabetic by a single intraperitoneal injection of streptozotocin (150 mg / kg body weight) and only those mice with serum glucose levels more than 350 mg/dl were used for transplantation.

Islet Isolation: Hamster islets were isolated according to the method previously described (9) by intraductal injection of collagenase solution followed by digestion and extensive purification on Ficoll gradients. Hand-picked islets were cultured in CMRL-1066 medium containing 10 % heat inactivated fetal calf serum (FCS) and antibiotic-antimycotic solution (1 ml/100 ml). The islets were then incubated at 37 ^{0}C for 3 days in a humidified atmosphere of 5 % CO_2. Islets were tested for viability by diphenylthiocarbazone (DTZ) stain.

Preparation of agarose microcapsules: Pancreatic islet microcapsules were prepared according to the method previously described [10]. Islets were washed by gravity sedimentation 3 times in CMRL-1066 medium containing only antibiotic-antimycotic solution. Agarose (Sigma Chemical Co., St.Louis, MO, USA) in phosphate buffer saline (PBS) without Ca and Mg salts at a concentration of 5 % was autoclaved and stored at room temperature. Before use, the gel was melted at 70^{0}C.then cooled to 40^{0}C and mixed with the islets suspended in an appropriate volume of growth medium. The mixture was poured into a 50 ml round- bottom glass centrifuge tube containing an equal volume of paraffin oil. The liquid was emulsified at room temperature with a vortex, to the desired bead size (~80-200 um). The mixing vessel was then cooled in an ice bath for 5 minutes and 50 ml of cooled growth medium were added. The tube was centrifuged at 1200 rpm for 10 minutes. The oil phase was removed by suction and 50 ml of growth medium was added. After mixing, the suspension was recentrifuged and the remaining oil was removed. The microcapsules were transferred to cultivation Petri dish containing complete CMRL-1066 medium with 10 % FCS and antibiotic-antimycotic solution (1ml/100ml). The microcapsules were then incubated overnight at 37 ^{0}C in a humidified atmosphere of air and 5 % CO_2.

Transplantation of microcapsules: At transplantation, a midline incision (2 mm) was made in the abdominal cavity and islet microcapsules were injected through 1 ml syringe without needle. A total of 40 mice were divided into 5 different treatment groups. Group 1 consisted of 5 mice grafted with 1000 free islets. Group 2 consisted of 5 mice grafted with 1000 free islets and 1000 empty agarose microcapsules. Group 3 consisted of 10 mice received 500 islet micocapsules. Group 4 consisted of 10 mice received 1000 islet microcapsules. Group 5 consisted of 10 mice received 1000 islet microcapsules that cultured in CMRL-1066 medium for 4 weeks at 37 ^{0}C.

Post-transplantation follow-up: After transplantation, the mice were transferred to metabolic cages for daily examination. Nonfasting blood glucose levels of the recipients were monitored 3 times weekly for the first 3 weeks, then twice weekly thereafter. The mice were considered cured if exhibited the following criteria: a random plasma glucose less than 200 mg/dl, aglucosuria and steady weight gain. Rejection was considered when the blood sugar concentration exceeded 200 mg/dl on two consecutive readings.

Glucose tolerance test: Glucose tolerance test was carried out in transplanted mice that maintained a normoglycemic state for at least 30 days as previously described [11]. For comparison, the glucose tolerance test was also performed to 5 normal non-transplanted mice and 5 diabetic non-transplanted mice. Glucose solution 1 mg/kg body weight was infused endogastrically through a polyethylene tube into mice that had been fasted overnight. Blood glucose levels were determined at 0,30,60,90 and 120 minutes after glucose injection. The results were expressed as mean blood glucose levels ± SD. The degredation in glucose level per minutes (k value) was calculated.

Stability of microcapsules: To assess the stability and islet preserving properties of microcapsules, groups of micocapsules (1000 microcapsules/group) were cultured in CMRL-1066 medium and incubated for 4 weeks at 37^{0}C in a humidified atmosphere of 5 % CO_2 (medium changed once per week). At the end of incubation period, the microcapsules were transplanted into diabetic mice.

Results

The islet microcapsules were purified by hand picking to be devoid of contamination as acinar cells, debris or empty microcapsules. Most microcapsules enclosed one islet and few enclosed two or more islets (fig. 1). The results of islet microcapsule transplantation are summarized in table 1. Mice transplanted with agarose pancreatic microcapsules (group 3,4,5) became normoglycemic and remained so for 30 to 65 days with gain in body weight. To confirm that encapsulation of islet xenografts in microcapsules prevented islet graft rejection, free hamster islets were transplanted intraperitoneally in 5 mice (group 1). None of these mice became normoglycemic. Similarly, when the hamster free islets were transplanted together with empty agarose microcapsules (5 mice, group 2), none of these mice became normoglycemic. To determine the ideal recommended number of islets required to achieve normoglycemia 500 or 1000 microcapsules were tried (10 mice / group). Although euglycemia was achieved in both groups, blood glucose readings were higher with flactuated values when less than 1000 microcapsules were used (fig. 2).

Table 1. Results of nonencapsulated and encapsulated islet transplants

No	*Transplanted group*	*Duration of normoglycemia (days)*	*Mean graft survival (days)*
1	1000 free islets	-----	-----
2	1000 free islets + 1000 empty capsule	-----	-----
3	1000 free islets + 1000 empty capsule	34,35,35,36,37,37,42,45,48,51	40±3
4	1000 encapsulated islets	30,42,44,44,50,53,55,59,62,65	50±3
5	1000 encapsulated and cultured islets	31,35,37,41,42,45,52,55,59,61	46±3

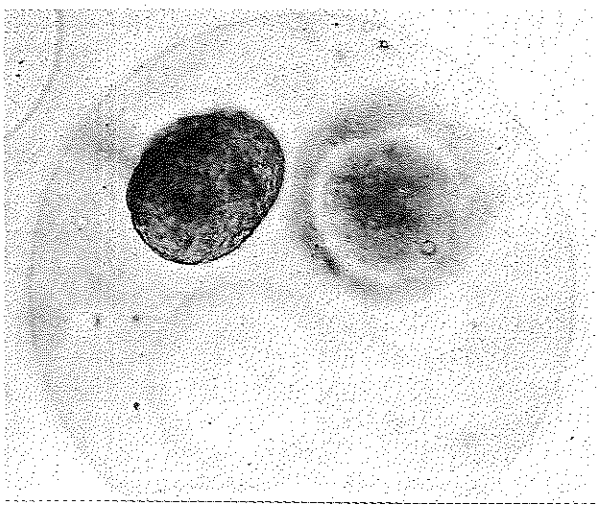

Fig. 1. Light photograph showing hamster islets encapsulated in agarose gel.

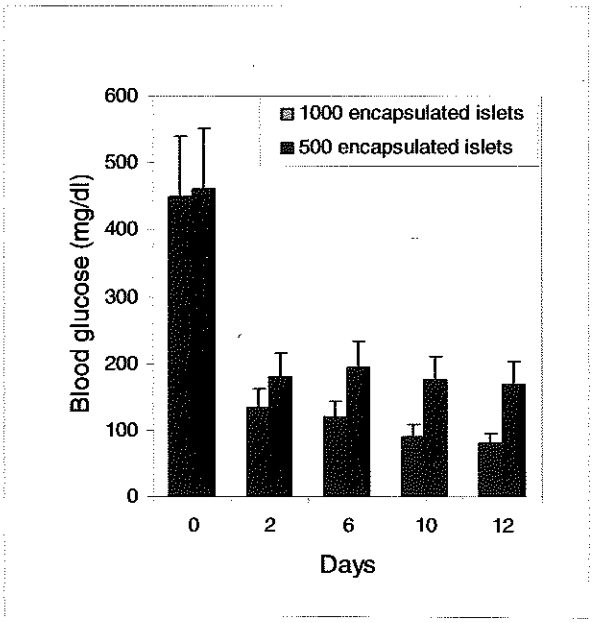

Fig. 2. The relation between blood glucose and days post-transplantation of cured mice.

The stability and islet preserving properties of microcapsules were evaluated in vitro (group 5). Groups of 1000 microcapsules were incubated for 4 weeks at 37 ^{0}C in CMRL-1066 medium. When these incubated microcapsules were transplanted into diabetic mice, all mice became euglycemic and maintained normoglycemia for more than 30 days. Glucose tolerance tests were carried out in transplanted mice that had maintained normoglycemic state for more than 30 days (fig 3). Five mice from each group were infused with 1 g / kg glucose after 30 days of transplantation. The mice were able to normalize their blood glucose. In normal mice and mice transplanted with islet microcapsules, plasma glucose peaked at 30 minutes and returned to base line levels by 120 minutes. Diabetic mice showed higher glucose levels after 120 minutes. Comparing the normal mice with transplanted mice, there was no significant difference in blood glucose level at 0, 30, 60, 90 and 120 minutes (fig. 3). These mice normalized their blood glucose and exhibited euglycemia 120 minutes after glucose infusion. The rate of glucose degradation as measured by the k value for the transplanted mice (2.5 ± 0.2) was higher than that of diabetic mice (0.85 ± 0.3). The k value for normal controls was (2.9 ± 0.2).

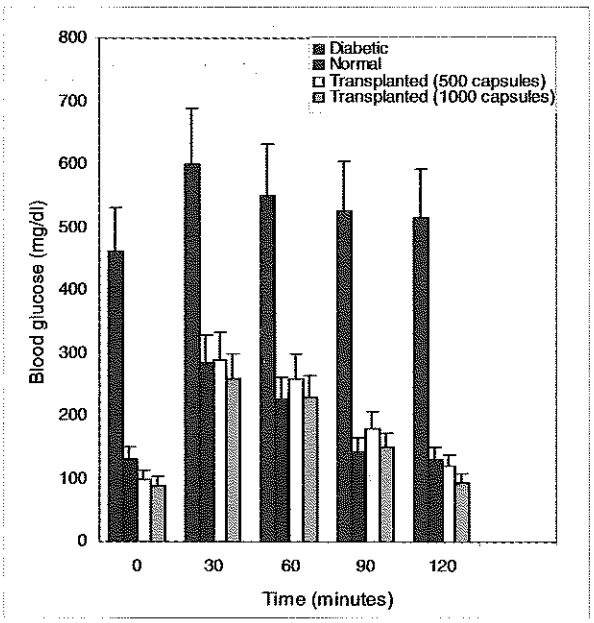

Fig. 3. Blood glucose levels in response to infused glucose (1g/kg) at 30 days following transplantation.

The microscopic examination of retained microcapsules 30 days after transplantation showed intact islets with neither tissue reaction nor adhesions (fig. 4). Moreover no evidence of inflammatory cells within or on the microcapsules were detected.

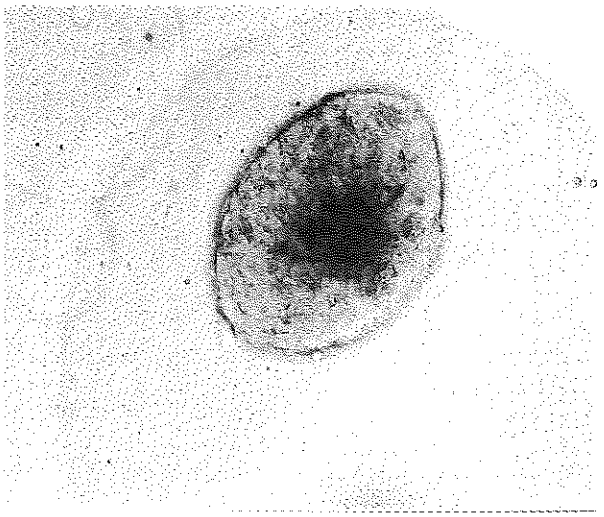

Figure 4. Light photograph showing microcapsule retained after 30 days of transplantation. There is no inflammatory cells within or on the microcapsule.

Discussion

Islets of langerhans have been considered extremely immunogenic, sometimes surviving only for one or more days if transplanted across a strong histocompatibility barrier [12]. A bioartificial pancreas has been proposed as a promising approach for treatment of insulin dependant diabetic patients [13]. The development of microencapsulation of xenogeneic islets in a semipermeable membrane is particularly attractive, since it might permit the use of xenografts to overcome the obstacle of scarcity of transplantable human pancreatic tissue without the need of any immunosuppression [14,15,16]. The data of our study showed that islets transplanted alone or with empty agarose microcapsules failed to normalize the blood glucose of the recipient mice, on the other hand, mice transplanted with agarose encapsulated islets enjoyed normoglycemia for maximum 65 days. Also when the microcapsules were cultured for 4 weeks before transplanted, all diabetic mice became normoglycemic. This indicates that these microcapsules continued to release insulin in the medium for 4 weeks and then continued to function in vivo. These data suggest that microcapsulation can be used as a method for the storage of islets prior to transplantation. Although successful encapsulated islet cells have been reported [16,17] there are still many reports of encapsulated islet graft failure due to pericapsular fibrosis [18]. In our study examination of encapsulated islet by light microscopy showed intact islets with no evidence of cellular infiltration around the microcapsule wall up to 4 weeks after transplantation. Our experiments demonstrate that agarose pancreatic islet microcapsules fulfill the properties required of an immunoisolatory device for pancreatic islet cell transplantation. These microcapsules are biocompatible, maintain viability and normal function of islets, prevent rejection, achieve normoglycemia in the recipient. Besides they can be easily and safely peritoneally implanted. It should also be emphasized that, despite the ability of the microcapsules to protect islets from immune destruction, the use of adequate number of highly purified viable islets is critical to achieving successful xenografting. In summary, the results of this study indicate that it is time to proceed with the development of microcapsules suitable for transplantation of islet xengrafts in larger animals and ultimately in humans. In addition, such microcapsules may be useful in treating conditions caused by an impaired functioning or the loss of other secretory cells, such as disorders of a growth factor or any other hormone. Also they may provide means for relatively long-term storage and preservation of islets prior to transplantation.

References

1- Lacy, P.: Status of islet cell transplantation. Diabetes Rev., 1993; 1 (1): 76.

2- De Vos, P., Hamel, A. and Tatarkiewicz, K.: Consideration for successful transplantation of encapsulated pancreatic islets. Diabetologia, 2002; 45 (2): 159.

3- Lim, F. and Sun, A.: Microcapsulated islets as bioartificial endocrine pancreas. Science, 1980; 210: 908

4- Soon-shiong, P., Feldman, E. and Nelson, R.: Long-term reversal of diabetes by the injection of immunoprotected islets. Proc. Natl. Acad. Sci. USA, 1993; 90: 5843.

5- Hobbs, H., Kendall, W., Darrabie, M. and Opera, E.: Prevention of morphological changes in alginate microcapsules for islet xenotransplantation. J. Investing. Med., 2001; 49 (6): 572.

6- Fan, M., Lum, Z., Fu.X., Levesque, L., Tai, I.., and Sun, A.: Reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets. Diabetes, 1990; 39: 519.

7- Scharp, D., Mason, N., and Sparks, R.: Islet immunoisolation: The use of hybrid artificial organs to prevent islet tissue rejection. World J. Surg., 1984; 8: 221.

8- Iwata, H., Takagi, T., and Amemiya, H.: Agarose for a bioartifical pancreas. J Biomed. Mater. Res., 1992; 26: 967.

9- Gotoh, M., Maki, T., and Kiyoiami, T.: An improved method for isolation of mouse pancreatic islets. Transplantation, 1985; 40 (4): 437.

10- Iwata, H., Kobayashi, K., Takagi, T., Oka, T., Yong, H., Amemiya, H., Tsuji, T. and Ito, F.: Feasibility of agarose microbeads with xenogeneic islets as a bioartificial Pancreas. J. Biomed. Mater. Res., 1994; 28 (9): 1003.

11- Jain, K., Yang, H., Cai, B., Haque, B., Hurvitz, A., Diehl, C., Miyata, T., Smith, B., Stenzel, K., Suthanthiran, M. and Rubin, A.: Retrivable replaceable macro-encapsulated pancreatic islet xenografts. Transplantation, 1995; 59 (3): 319.

12- Barker, C., Naji, A. and Siluvs, W.: Immunologic problems in islet transplantation. Diabetes, 1980; 29: 86.

13- Prokop, A.: Bioartificial pancreas: Materials, devices, function and limitations. Diab. Technol. Ther., 2001; 3 (3): 431.

14- Schneider, S., Feilen, P., Slotty, V., Kampfner, D., Preuss, S., Berger, S., Beyer, J. and Pommersheim, R.: Multilayer capsules: a promising microencapsulation system for transplantation of pancreatic islets. Biomaterials, 001; 22 (14): 1961.

15- Mullen, Y., Maruyama, M. and Smith, C.: Current Progress and perspectives in immunoisolated islet transplantation. J. Hepatobiliary Pancreat. Surg., 2000; 7 (4): 347.

16- Sun, A., Fan, M., Lum, Z., Fu X., Noton, J.: Bioartificial pancreas: reversal of diabetes in BB rats by transplantation of encapsulated pancreatic islets. Artif. Organs, 1989; 13:567.

17- Takagi, T., Iwata, H., Kobayashi, K., Oka,T., Tsuji,T.,and Ito, F.: Development of a microcapsule applicable to islet xenotransplantation. Transplant. Proc., 1994; 26 (2): 801.

18- Wijsman, J., Atkison, P., Mazaheri, R., Garcia, B., Paul, T., Vose, J., Oshea, G., and Stiller, C.: Histological and immunopathological analysis of recovered encapsulated allogeneic islets from transplanted diabetic BB/W rats. Transplantation, 1992; 54: 588.